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A Papillomatous Disease of the Gallbladder Associated with Infection by Flukes, Occurring in the Marine Turtle, *Chelonia mydas* (Linnaeus).[1]

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(Plates I–IV; Text-figure 1).

In two earlier publications attention was called to certain cutaneous tumors occurring in the turtle *Chelonia mydas* (Linnaeus) (Smith & Coates, 1938, 1939). These growths, fibro-epithelial in character, are found distributed on the neck and in the axillary, inguinal, eyelid, conjunctival and corneal regions. As a rule the tumors have the morphological characteristics of benign growths; rarely does the structure suggest a malignant change. In the majority of such tumors ova of a blood fluke can be demonstrated on microscopical study, giving rise to the hypothesis that blood flukes or ova may act as causative factors in the production of these lesions. In earlier studies ova occurring in cutaneous tumors were identified as those coming from *Hapalotrema constrictum* (Leared). Recent studies of a number of these worms (Nigrelli, 1940), however, indicate that this blood fluke is the same as *Distomum constrictum* of Leared (1862) and the form described by Price (1934) as *Learedius learedius*. The form described as *Hapalotrema constrictum* (Leared) Looss, 1899 (= *Mesogonimus constrictum* Monticelli, 1896) is now designated as *Hapalotrema mistroides* (Monticelli). This form is also a blood fluke but found in another species of marine turtle *Caretta caretta* (Linnaeus) (= *Thalassochelys caretta* (Linnaeus)). It must be pointed out here, however, that the original identification was tentative and based on the shape and size of the eggs, which are strikingly similar. Relationships and identifications of worms have been based on egg characteristics by various taxonomists.

Further studies of lesions in turtles as a result of fluke infections, carried on during the past year, have revealed a new form of papillomatous disease of the gallbladder of *Chelonia mydas* which may be the result of a fluke infection of this organ.

A brief description of the gallbladder lesions and of the fluke itself follows.

Infected gallbladders show a wide range of lesions, depending doubtless upon the intensity and duration of the infection and the susceptibility of the tissues of the host. Any part of the fundus of the gallbladder may show thickened papillomatous change in solitary patches (Plate I, Fig. 1; Plate II, Fig. 4) or in confluent irregular masses (Plate I, Fig. 2). A papillomatous hyperplasia of the mucous membrane near the cystic duct's entrance into the gallbladder may be the most conspicuous lesion, the result of infection by trematodes (Plate II, Figs. 4, 5, 6). In consequence, the cystic end of the gallbladder may appear greatly thickened and encroaching on the lumen of the gallbladder (Plate IV, Fig. 12) almost to the point of producing a stenosis or obstruction. At times the entire wall of the gallbladder participates in the lesion. In these circumstances the epithelium is thrown up into papillomatous folds (Plate III, Figs. 7, 8), the muscularis shows increased amounts of connective tissue and lymphoid cell infiltration and the subserosa may be oedematous, thickened, and contain a large number of dilated capillaries both vascular and lymphatic. All sorts of irregularly dilated glands are formed in the hyperplastic epithelium. Often glands penetrate down into the submucosa. However, malignant invasive changes have not been noted to date in approximately one hundred gallbladders examined which have shown the effects of fluke infection. Malignant change might readily be expected as a result of these chronic hyperplastic changes. In order to determine whether or not the papillomatous lesions do become malignant it will be necessary to have

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access to more extensive material than is available at the present time.

The majority of the flukes lie free in the dark green, thickened, often inspissated bile of the infected gallbladder. Flukes often are attached to the papillomatous parts of the mucous membrane (Plate IV, Fig. 11). The parasites may lie partly buried in the mucosa (Plate I, Figs. 1, 2). There can be little doubt that the presence of flukes at the site of characteristic lesions of the mucous membrane indicates that the underlying cause of the disease arises in mechanical or chemical factors related to the activities of the infecting flukes. Ova surrounded by epithelioid or even giant cells may be found in the mucous membrane or other parts of the walls of the gallbladder (Plate III, Fig. 9). Mucous production is abundant (Plate III, Fig. 10).

The parasites from the gallbladder of *Chelonia mydas* have been identified as *Rhytidodoides similis* Price, 1939. A related species *R. intestinalis* was described in the same paper. According to Price, the identifications of "Both *R. intestinalis* and *R. similis* are in each case based on only two specimens; consequently it is not possible to determine the amount of variation within the species." A collection of large numbers of the gallbladder form has afforded an opportunity to make just such a study for this species, at least (Text-figure 1).

The worms are lanceolate in shape, tapering at both ends of the body and broadening considerably in the middle region. The posterior tip of the body can be extended into a minute "tail-like" appendage. When the flukes are examined in profile there is a characteristic hump on the dorsal side. In contracted specimens, the hump is more pronounced than in the extended forms. On the ventral surface, the acetabulum is prominent and immediately anterior to this sucker may be seen the genital ridge and pore.

The flukes are transparent and stained with bile. They measure 1.2 × .42–4.4 × 1.8 mm., with an average size of 2.2 × .97 mm. for fifty specimens. Cuticula without spines, smooth in expanded worms, with minute folds in contracted specimens. Oral sucker subterminal, measuring 140.2–342 microns in diameter (average, 293.4 microns), with lateral projections. The projections are prominent in the smaller individuals, less so in larger forms but are nevertheless a constant feature. Acetabulum, 170.3–385.6 microns (average, 300.7 microns), pre-equatorial. Pre-pharynx lacking; pharynx 80–179 × 50–170 microns; esophagus of variable length, depending entirely on the state of contraction and expansion of the worms. Intestinal ceca simple, extending to the posterior extremity of the body. Excretory pore subterminal, dorsal; excretory vesicle Y-shaped, branching immediately behind the posterior testis, with the arms extending as far anterior as the pharynx. Genital aperture median, anterior to the ventral sucker and in the region of the cecal bifurcation. Cirrus pouch elongate piriform, muscular, situated over or slightly in front of the acetabulum, measuring 150–700 microns × 150–456 microns (average measurement 152 × 478.8 microns). Seminal vesicle and *pars prostatica* present. Testes in posterior third of the body, placed one behind the other or slightly oblique to the axis of the body, sometimes globular, sometimes sub-globular or ovoid in shape. Anterior testis usually smaller, measuring 102–420 microns × 250–456 microns; posterior testis 152–532 microns in diameter. In very small forms testes and vitellaria are poorly developed. Ovary globular, 91–280 microns in diameter (average, 250 microns), sub-median and pre-testicular in position. Ovarian complex, consisting of Mehlis' gland, seminal receptacle, vitalline reservoir, and Laurer's canal. Vitellaria consist of four groups of more or less elongated follicles; two small groups of follicles are in the anterior region of the body and present as two distinct masses, one on each side of the esophagus between the acetabulum and pharynx; the two larger groups extend from the acetabulum to the posterior extremity of the body. Main vitelline ducts pass medially into the ovarian complex at about the level of the posterior border of the ovary. Uterus fills the entire intercecal space between the ovary and acetabulum; metraterm present. Eggs yellowish, thick-shelled, embryonated, measuring on the average 36 × 72 microns.

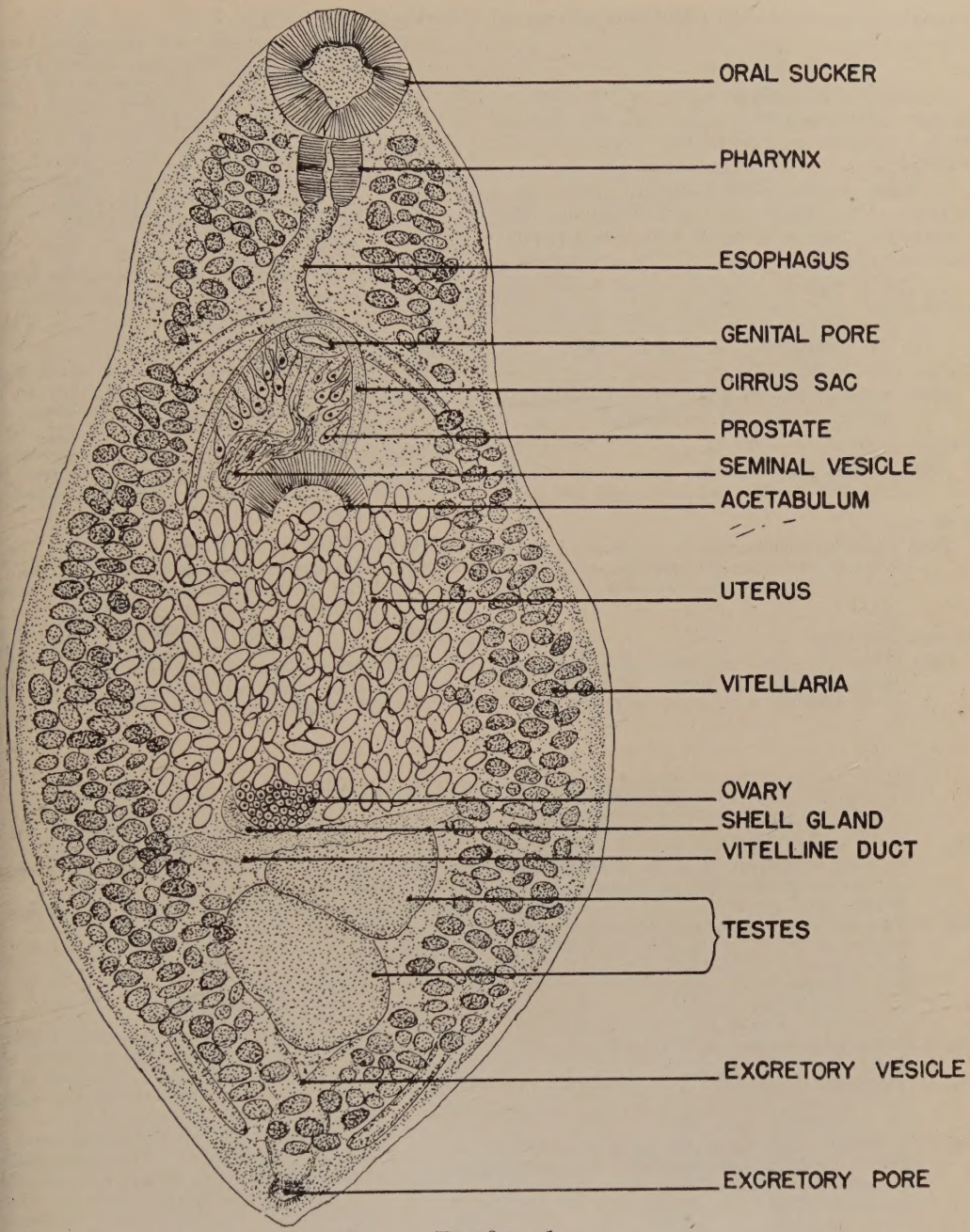
Comment.

The histo-pathology of trematode infections among lower vertebrates is not well known. The condition reported above is in many respects not unlike liver fluke infections in mammals (see Craig & Faust, 1940).

In sheep liver fluke, *Fasciola hepatica* Linnaeus, larvae are ingested with contaminated vegetation. Excystment takes place in the duodenum where the parasites penetrate the wall and pass into the body cavity. They continue their migration, passing through the liver capsule directly into the liver substance. The mechanical irritation produced, in the case of heavier infections, results in necrosis and fibrosis of the liver. Such an infection in man may cause cystic enlargement of the ducts, adenomata of the biliary epithelium, leucocytic and eosinophilic infiltration, and eventually the development of scar tissue. Often, in their wanderings through the liver substance, the worms produce abscesses, and the mechanical damage causes atrophy of the liver and the portal vessels.

Local lesions of this kind may be produced by other liver flukes such as the lancet fluke, *Dicrocoelium dentriticum* (Rudolphi), the cat liver fluke, *Opisthorchis felineus* (Rivolta), and the Chinese liver fluke, *Clonorchis sinensis* (Cobbold).

In dicrocoeliiasis, sheep and other herbiverous mammals become infected by eating grass and other vegetation containing encysted larvae. When eaten, such larval forms excyst in the duodenum and find their way to the biliary passages, often producing hypertrophy of the



Text-figure 1.

Rhytidodoides similis Price. × 54.

epithelium. Both opisthorchiasis and clonorchiasis infections are brought about by eating fish poorly cooked, improperly cured, or raw, harboring the encysted stage.

In clonorchiasis, the larvae, following excystment, make their way up the common bile duct and migrate to the distal bile capillaries. Lesions produced by such infections have been described by Faust & Khaw (1927), Hoeppli (1933) and others. These include the proliferation of biliary epithelium, crypt formation in the bile duct, periepithelial fibrosis, periportal connective tissue hyperplasia and fibrous development around masses of eggs infiltrated in the liver substance.

Although the papillomatous disease of the gallbladder of *Chelonia mydas* is stressed in the present contribution, there are also definite responses in the liver and biliary ducts of these

animals which will be discussed in a subsequent report.

SUMMARY.

1. Gallbladders of the marine turtle *Chelonia mydas* (Linnaeus) were found heavily infected with a fluke referred to as *Rhytidodoides similis* Price, 1939.

2. These flukes produce certain pathological changes in the tissues of the gallbladder. The outstanding feature of these lesions is a papillomatous hyperplasia of the mucous membrane.

3. The parasites inducing these lesions are redescribed.

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EXPLANATION OF THE PLATES.

PLATE I.

Fig. 1. Strip of gallbladder mucous membrane showing to the right a small papilloma with several flukes attached to or partly buried in the mass.

Fig. 2. Photograph of inside of gallbladder attached to the liver. Note confluent masses of papillomatous tissue; flukes lie attached or partly buried in the growth.

PLATE II.

Fig. 3. Section of normal gallbladder containing bile.

Fig. 4. Thickened, shrunken, infected gallbladder with several small papillomata near the entrance of the cystic duct.

Figs. 5–6. Small contracted gallbladders infected by fluke, *Rhytidodoides similis*. The great thickening of the mucosa and submucosa has occurred near the cystic duct.

PLATE III.

Figs. 7–8. Irregular hyperplasia of papillomatous regions of infected gallbladders. × 35.

Fig. 9. Photo-micrograph of papillomatous region of gallbladder showing fragments of ova at points **a** and **b**. Epithelioid cells surround the small fragment at **b**. × 120.

Fig. 10. Masses of mucous formed at the surface of papilloma. × 100.

PLATE IV.

Fig. 11. Microscopic section of trematode *Rhytidodoides* attached to the hyperplastic epithelium of the gallbladder. Section of the parasite is at the level of the ventral sucker and the cirrus sac. × 40.

Fig. 12. Photo-micrograph of the cystic end of infected gallbladder. The hyperplastic mucous membrane is thrown into countless folds which encroach upon the lumen of the gallbladder. Submucosa and muscularis are thickened. The subserosa seen in the upper part of the photograph is oedematous and contains numerous blood and lymphatic vessels. × 25.

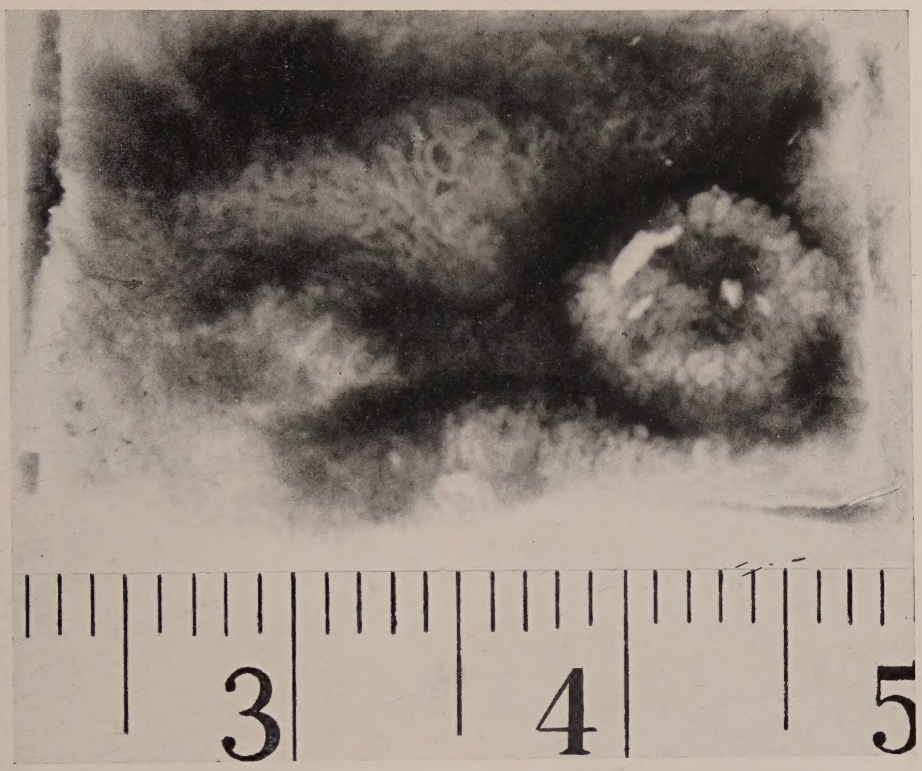


FIG. 1.

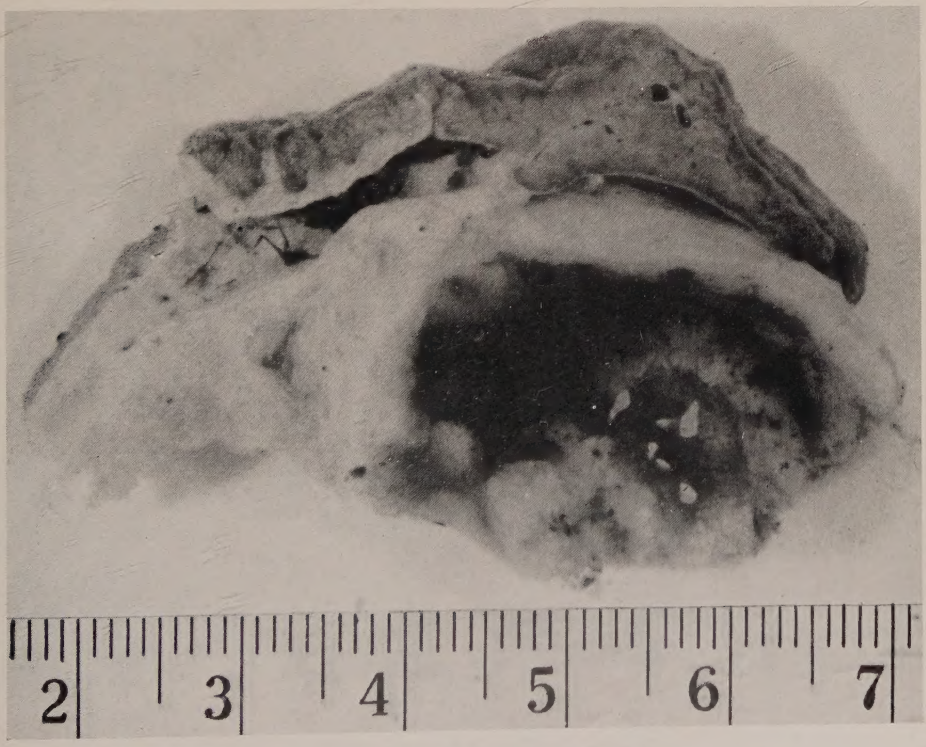
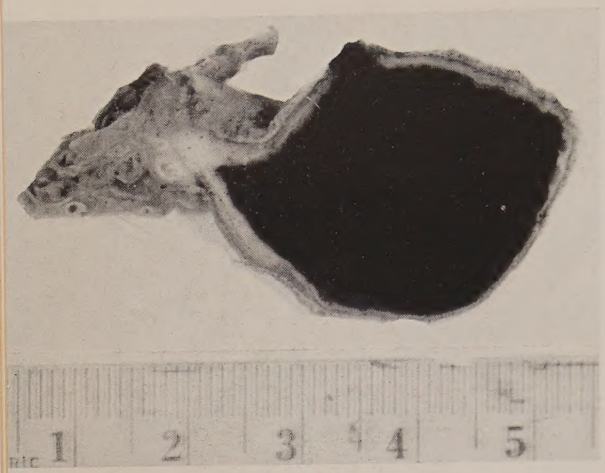


FIG. 2.

A PAPILLOMATOUS DISEASE OF THE GALLBLADDER ASSOCIATED WITH INFECTION BY FLUKES, OCCURRING IN THE MARINE TURTLE, CHELONIA MYDAS (LINNAEUS).



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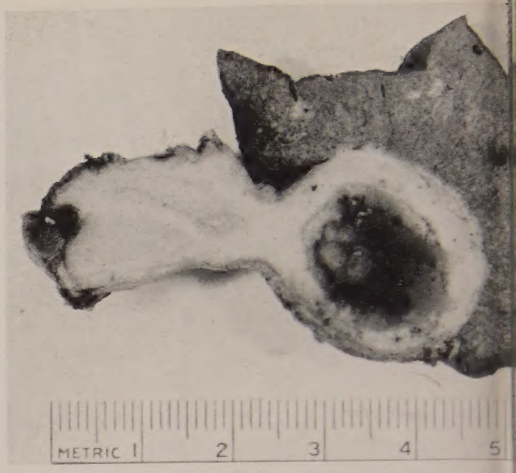

FIG. 4.

FIG. 5.

FIG. 6.

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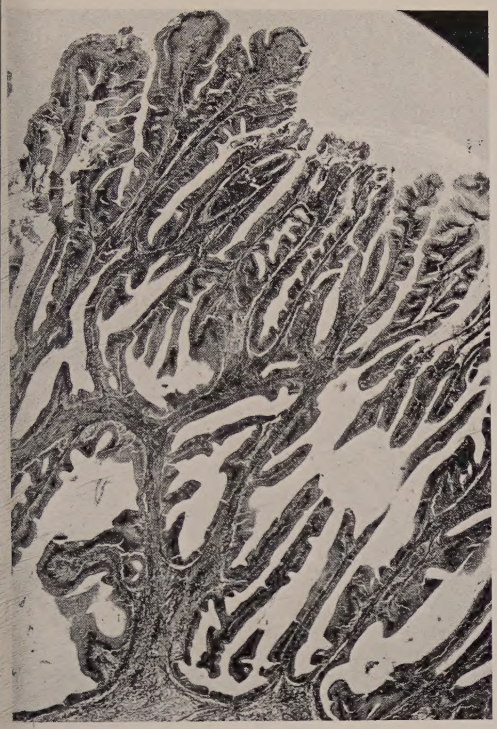

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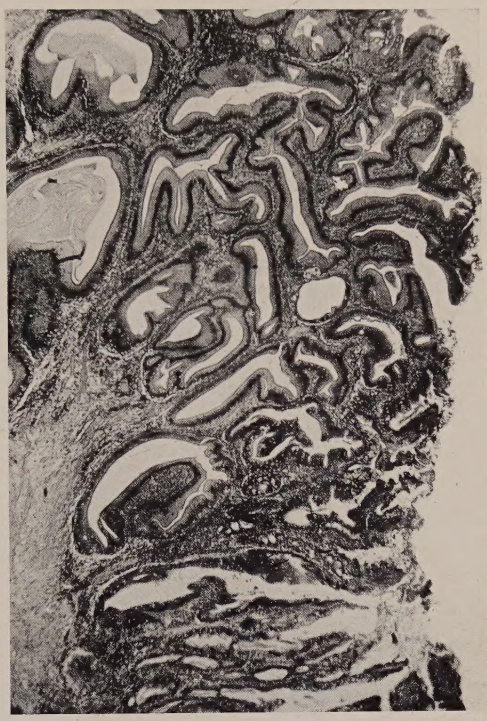

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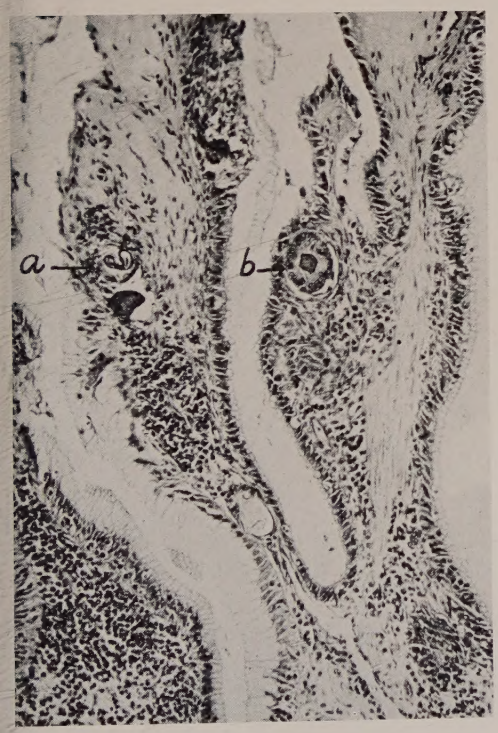

FIG. 9.

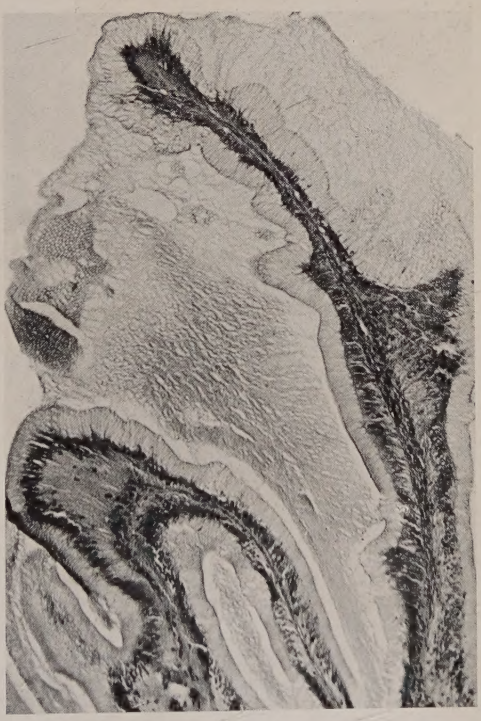

FIG. 10.

A PAPILLOMATOUS DISEASE OF THE GALLBLADDER ASSOCIATED WITH INFECTION BY FLUKES, OCCURRING IN THE MARINE TURTLE, CHELONIA MYDAS (LINNAEUS).

FIG. 11.

FIG 12.

A PAPILLOMATOUS DISEASE OF THE GALLBLADDER ASSOCIATED WITH INFECTION BY FLUKES, OCCURRING IN THE MARINE TURTLE, CHELONIA MYDAS (LINNAEUS).